NEURAL RESPONSES TO INJURY: PREVENTION, PROTECTION, AND REPAIR
Annual Technical Report
1994

Submitted by

Nicolas G. Bazan, M.D., Ph.D.
Project Director

Period Covered: 20 September, 1993, through 19 September, 1994

Cooperative Agreement DAMD17-93-V-3013

between

United States Army Research and Development Command
(Walter Reed Army Institute of Research)

and

Louisiana State University Medical Center
Neuroscience Center of Excellence

REPAIR AND REGENERATION OF PERIPHERAL NERVE DAMAGE

Project Directors:

Roger Beuerman, PhD
David Kline, MD
Austin Sumner, MD

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6. AUTHOR (S)
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Professor of Ophthalmology, Biochemistry and Molecular Biology and Neurology

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LSU Neuroscience Center
2020 Gravier Street, Suite B
New Orleans, LA 70112

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13. ABSTRACT *(Maximum 200 words)*

The LSU Neuroscience Center is a comprehensive, multidisciplinary, and transdepartmental entity that unites fundamental neurobiology and the clinical neurosciences in the common goal of elucidating the workings of the brain and contributing to the treatment of currently incurable diseases of the nervous system. The objective of the present program is to find solutions to neuroscience-related problems of interest to the U.S. Army Medical Research and Development Command. The program is focused on exploiting novel neuroprotective strategies that lead to prevention of and repair after neural injury. Converging approaches using state-of-the-art tools of cell biology, neurochemistry, neuroimmunology, neurophysiology, neuropharmacology, molecular biology and virology are proposed. Over the next four years, this program aims to: 1) carry out seven research projects in the basic and clinical neurosciences; 2) expand central, shared facilities with the addition of highly specialized instrumentation not currently available to our scientists; 3) develop laboratory space to permit the physical consolidation and coordination of this research effort; and 4) institute a coordination unit to monitor, facilitate, and administrate the cooperative research programs, as well as to meet the associated budgetary, human resources, facilities, and communications needs for the attainment of the proposed program goals.

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This Technical Report covers the progress made in the first year of this Cooperative Agreement in one project of the original proposal. We hope that this format of the report will facilitate its handling. The table of contents for all the projects has been included in each volume as well as letters from members of the External Advisory Committee of the LSU Neuroscience Center who have conducted an initial review of the work done supported by this Cooperative Agreement.

Nicolas G. Bazan, M.D., Ph.D.
Director, LSU Neuroscience Center
Program Director, USAMRDC Cooperative Agreement

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Abstracts: Psychoneuroimmunology Research Society

1. HSV-1 latently-infected mice display an altered response to stress: Implications for antiviral immunity.
2. Mouse lymphocytes express an orphan opioid receptor
3. Morphine suppresses peritoneal and splenic CTL activity in a dose-dependent fasion in alloimmunized mice
4. The frequency of exposure to morphine differentially affects CTL activity in alloimmunized mice.

Manuscripts:

1. Carr DJJ, Carpenter GW, Garza HH, Baker ML, Gebhart BM (in press) Cellular mechanisms involved in morphine-mediated suppression of CTL activity. In: *The Brain Immune Axis in Substance Abuse* (Sharp, Friedman, Maddin and Eisenstein, eds), Plenum Press.
2. Carpenter GW and Car DJJ (submitted) Pretreatment with β-funaltrexamine blocks morphine-mediated suppression of CTL activity in alloimmunized mice.
3. Carr DJJ and Carpenter GW (submitted) Morphine-induced suppression of spenic CTL activity in alloimmunized mice is not mediated through a δ-opioid receptor.
4. Carpenter GW, Garza HH, Gebhardt BM, Car DJJ (in press) Chronic morphine treatment suppresses CTL-mediated cytolysis, granulation and cAMP responses to alloantigen

Project Director: Nicolas G. Bazan, M.D., Ph.D.

Participating Scientists: Geoffrey Allen, Ph.D.
Gary D. Clark, M.D.
Victor Marcheselli, M.S.
John Hurst, Ph.D.
Leo Happel, M.D.
Walter Lukiw, Ph.D.

Roger Beuerman, Ph.D.

Participating Scientists: Claude A. Burgoyne, M.D.
Emily Varnell
Mandi Conway, M.D.

Manuscripts

1. Chew SJ, Beuerman RW, Kaufman HE (in press) Real-time confocal microscopy of keratocyte activity in wound-healing after cryoablation in rabbit corneas. *Scanning* 16.

"Role of Growth Factors and Cell Signaling in the Response of Brain and Retina to Injury" . . . . .

Project Directors: Prescott Deininger, Ph.D.
Nicolas G. Bazan, M.D., Ph.D.

Participating Scientists: Julia Cook, Ph.D.
Haydee E. P. Bazan, Ph.D.
William C. Gordon, Ph.D.
Elena Rodriguez De Turco, Ph.D.
Victor Marcheselli, M.S.

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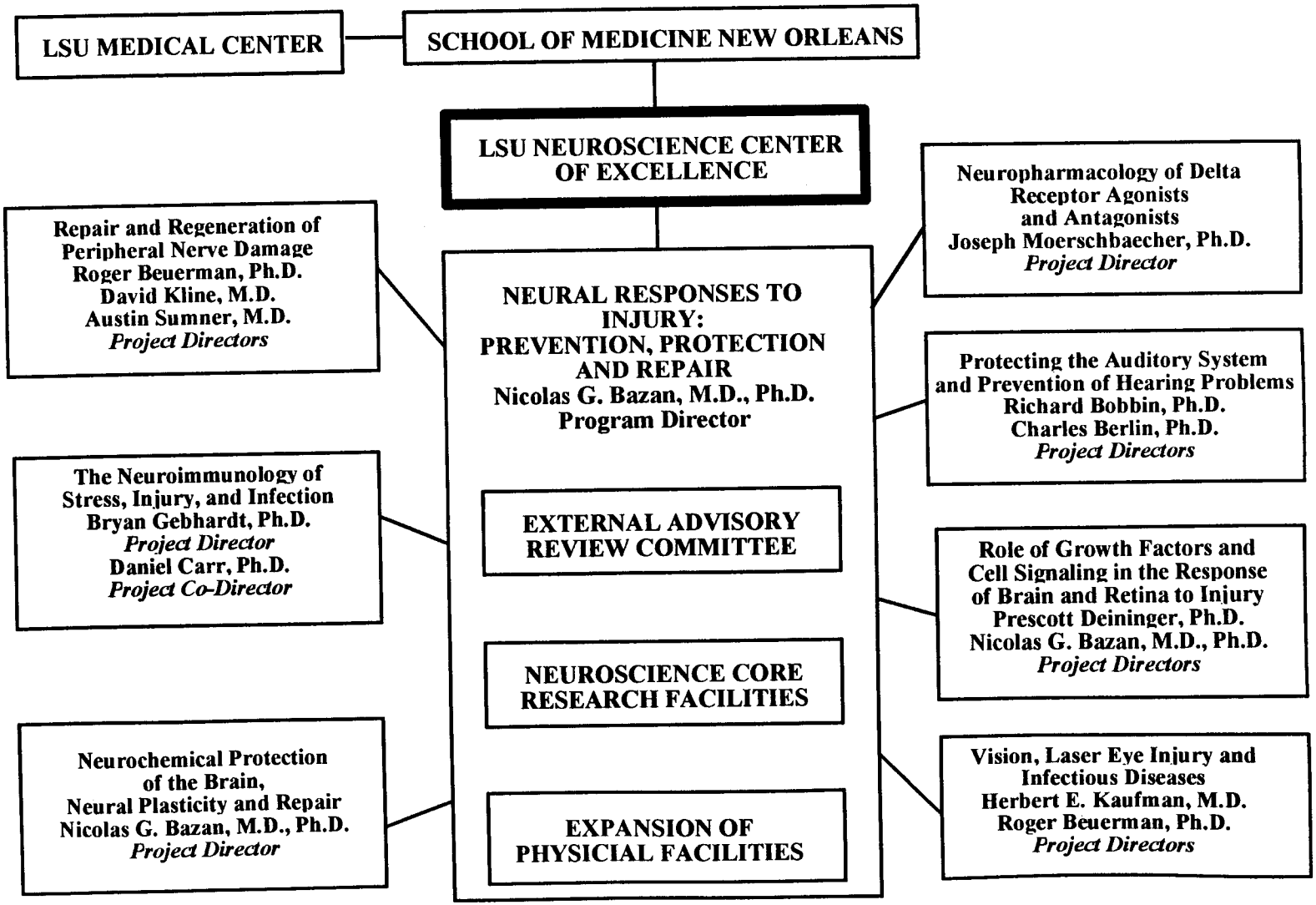
Cooperative Agreement Between the US Army Medical Research and Development Command and The LSU Neuroscience Center of Excellence

DAMD17-93-V-3013 20 September, 1993 - 19 October, 1997 $13,860,000



SCHOOL OF
MEDICINE IN NEW ORLEANS
Louisiana State University
Medical Center
2020 Gravier Street, Suite "B"
New Orleans, LA 70112-2234
Telephone: (504) 568-6700
Telefax: (504) 568-5801

Neuroscience Center
Office of the Director

19 October, 1994

Commander
U.S. Army Medical Research and Development Command (USAMRDC)
ATTN: SGRD-RMI-S
Fort Detrick
Frederick, MD 21702-5012

Re: Annual report, Cooperative Agreement No. DAMD17-93-V-3013
Neural Responses to Injury: Prevention, Protection, and Repair

Dear Sir,

Please find enclosed the original and five copies of the first annual report for the Cooperative Agreement, referenced above, between the USAMRDC and the Louisiana State University Medical Center School of Medicine, Neuroscience Center of Excellence. This report represents the research carried out during the first year of this agreement (20 September, 1993, to date). It is organized per project, each corresponding to a chapter of the original application.

In addition to the research conducted in the first year of this agreement, the planning for the two additional floors of research space which are to be added to the Lions/LSU Clinics Building, 2020 Gravier Street, New Orleans, LA, has been completed, including all specifications necessary for the start of bidding. Enclosed is one copy each of the program manual (1 vol.) and the project manual (3 vols.) which has been generated by Cimini, Meric and Duplantier, Architects and Planners, for bidding purposes. It should be noted that there will actually be three floors constructed in this one project, two as funded by this Cooperative Agreement and one which is funded by LSU to be used by the School of Medicine for other purposes.

As planned, I arranged to have three meetings between the LSU investigators and their counterparts in the Army to provide program briefings for the work that they were planning to conduct under this agreement as well as to exchange ideas and information of mutual interest. The agendas for each of these meetings are enclosed. These provided both the LSU scientists and those of the Army the opportunity to discuss the work being done, the direction, and the significance to problems of interest to the Department of Defense.

On 2 December, 1993, several of our investigators, excluding the Auditory and Laser/Vision groups, met at the Walter Reed Army Institute of Research, Washington, D.C., with Drs. Frank Tortella, Joseph Long, Mark DeCoster and Jit Dave. These discussions revolved around the neurochemical and neuropharmacological aspects of the program project and provided a forum for the Army scientists tobegin interactions and exchange of information with our investigators.

On 31 January, 1994, the LSU auditory physiology group, represented by Drs. Charles Berlin and Richard Bobbin, and I met at Fort Rucker, AL, with Dr. Kent Kimball and Dr. Ben T. Mozo. These meetings involved presentations and discussions about the protection of the auditory system and prevention of hearing problems in humans.

The LSU investigators involved with the vision research, composed of Dr. Herbert Kaufman, Dr. Roger Beuerman and myself, met on 7 February, 1994, at Brooks Air Force Base, San Antonio, TX. These scientists and those of the Ocular Hazards Research Unit of the US Army Medical Research Detachment made presentations and conducted discussions focused on protection from, repair of, and prevention of laser injuries, specifically to the eye. Each of these information exchanges provided very useful direction and advice for the LSU investigators. These workshops will be conducted annually for the term of this agreement.

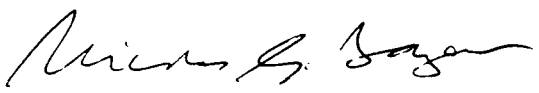
At the end of the first year of this program, as planned, I requested that two of the members of the External Advisory Committee of the LSU Neuroscience Center, Dr. Dennis W. Choi, Jones Professor and Head of the Department of Neurology, Washington University School of Medicine, and Dr. Fred Plum, Anne Parrish Titzell Professor and Chairman of the Department of Neurology, Cornell University Medical College, provide a critical review and a written report of the progress of the research accomplished under this Cooperative Agreement. Dr. Choi was given a copy of this annual report and subsequently made a site visit on 15 September, 1994, to the LSU Neuroscience Center. (The agenda for his meeting is attached.) At that time he met with a number of the investigators and administrators involved with whom he discussed many facets of the research being performed under this Agreement. His opinion of the work being done is attached.

Dr. Fred Plum made a site visit on 26 September, 1994, having also been provided previously with a copy of this annual report. He was also given the opportunity to examine the research and other progress made under this agreement and his written critique is also attached. Please note that, near the end of his letter (bottom of page two, first four paragraphs of page 5), Dr. Plum also included a description of projects not directly supported by the Cooperative Agreement but which are very positively impacted by any support of Neuroscience projects. The

reviewers were very complimentary of the positive consequences resulting from this support.

We are very pleased with the progress that has been made. We would like to thank you for the assistance you have given us. Please let me know if there is any further information that I can provide you.

Sincerely,



Nicolas G. Bazan, M.D., Ph.D.
Villere Professor of Ophthalmology,
Biochemistry and Molecular Biology,
and Neurology
Director, LSU Neuroscience Center

NGB/eht
enclosures

JOINT WORKSHOP ON "NEURAL RESPONSES TO INJURY: PREVENTION, PROTECTION AND REPAIR"

Sponsored by the LSU Neuroscience Center and Walter Reed Army Institute of Research, Department of Medical Neurosciences

December 2, 1993
Building 40, Room 2133

"Overview of LSU Program" **N. Bazan**	9:00
"Repair and Regeneration of Peripheral Nerve Damage" **R. Beuerman, D. Kline, J. England**	9:20
"The Neuroimmunology of Stress, Injury and Infection" **D. Carr**	10:10
Break	10:20
"Neurochemical Protection of the Brain, Neural Plasticity and Repair" **N. Bazan**	10:40
"Neuropharmacology of Delta Receptor Agonists and Antagonists" **J. Moerschbaecher**	11:15
"Stress and the Dopamine System" **J. Rao**	11:45
Box Lunch Served ($2.00 each)	12:00
"Role of Growth Factors and Cell Signaling in the Response of Brain and Retina to Injury **N. Bazan and J. Cook**	12:10
"An Overview of Neuropharmacology Research at WRAIR on Nervous System Injury and Protection" **Frank Tortella**	13:00
"Animal Models of Spinal Cord Injury and Mechanisms of Blood Flow Changes" **Joseph Long**	13:30
"Evaluation of Excitatory Amino Acids in Neuronhal Cell Culture" **CPT DeCoster**	13:50
"Molecular Biology of Nervous System" **Jit Dave**	14:10
Overall Discussion	14:30
Adjourn	15:00

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Joint Workshop on Neural Responses to Injury:
Prevention, Protection and Repair
Walter Reed Army Institute of Research, Dept. of Medical Neuroscience
U.S. Army Aeromedical Research Laboratory, Fort Rucker, AL
SCHEDULE FOR JANUARY 31, 1994

January 30
12:00 PM - depart New Orleans by car
Hotel: **Comfort Inn, 615 Boll Weevil Circle, Enterprise, AL 36330**
Tel. 205-393-2304, Fax. 205-347-5954

January 31
Visiting - Dr. Kent Kimball, Director, Plans and Programs, USAARL
Dr. Ben T. Mozo, Research Physicist, USAARL
Fort Rucker, AL 36362-5292
Tel. (205) 255-6917, Fax. (205) 255-6937

9:00 AM - Welcome
9:20 AM - Overview of LSU Program - **Nicolas G. Bazan**
9:45 AM - Protection the Auditory System and Prevention of Hearing Problem via Efferent Activation in Humans - **Charles Berlin**
10:30 AM - Break
11:00 AM - Prevention of Hearing Problems in Animals - **Richard Bobbin**
12:00 PM - General Discussion and Lunch
13:00 PM - Adjourn

OCULAR HAZARDS RESEARCH
U.S. ARMY MEDICAL RESEARCH DETACHMENT
7914 A DRIVE (Bldg 176)
BROOKS AIR FORCE BASE, TEXAS 78235-5138

February 7, 1994

Leave New Orleans on Continental flight #1445 at 6:00 PM, arrive San Antonio on Continental flight #1120 at 8:53 PM.

Hyatt Regency San Antonio
123 Losoya St., San Antonio, TX 78205
Confirmation #HY0000605552

February 8, 1994

8:30 *Overview of USAMRD program*
Bruce Stuck, Director, USAMRD

8:45 *Review of Accidental Laser Exposures and Human Tissue Response*
Donald Gagliano, Commander, USAMRD

9:00 *Overview of LSU Program*
Nicolas G. Bazan, Director, LSU Neuroscience Center

9:10 *The Program: Vision, Laser Eye Injury, and Infectious Diseases*
Herbert Kaufman, Chairman, Ophthalmology Dept. LSU

10:00 *Confocal Approach to Cellular Reactions in Wound Healing and of the Lamina Cibrosa.*
Roger Beuerman of the LSU Neuroscience Center

10:30 **BREAK AND LAB TOUR**

10:50 *Neurochemical Protection of the Brain, Neural Plasticity, and Repair*
Nicolas Bazan, Director, LSU Neuroscience Center

11:40 *Basic Fibroblast Growth Factor (bFGF) Treatment of Laser-Injured Retina*
Steven T. Schuschereba, Chief, Biology Section, USAMRD

12:10 *Role of Growth Factors and Cell Signaling in the Response of Brain and Retina to Injury: Focus on the Retina*
Nicolas Bazan, Director, LSU Neuroscience Center

12:50 **LUNCH**

2:50 Depart San Antonio on Southwest flight #803

5:55 Arrive New Orleans on Southwest flight #1055

LETTERS FROM MEMBERS OF THE EXTERNAL ADVISORY COMMITTEE

WASHINGTON UNIVERSITY SCHOOL OF MEDICINE
AT WASHINGTON UNIVERSITY MEDICAL CENTER

NEUROLOGY

Dennis W. Choi, M.D., Ph.D.

Andrew B. and Gretchen P. Jones Professor and Head
Neurologist-in-Chief, Barnes Hospital

October 17, 1994

Nicholas G. Bazan, MD, PhD
Director, LSU Neuroscience Center
School of Medicine in New Orleans
Louisiana State University Medical Center
2020 Gravier Street, Suite "B"
New Orleans, LA 70112-2234

Dear Nick:

Thank you for the invitation to visit LSU on September 15 and review early progress made under the LSU Neuroscience Center of Excellence Cooperative Agreement with the U.S. Army Medical Research and Development Command.

You have assembled an impressive array of faculty researchers to study diverse aspects of nervous system injury. Overall, I find the individual projects to be thoughtful and well chosen. With you as director, I am sure that they will be most ably integrated. Your project 3 "Neurochemical Protection of the Brain, Neuroplasticity and Repair" is in my view the clear focal point of the overall program. The identification of new PAF antagonist drugs capable of regulating excitatory synaptic transmission and excitotoxic central nervous system injury, is an attractive and attainable goal. The novel pharmacology theme is also well developed in Dr. Moerschbaecher's Section 4 "Neuropharmacology of Delta Receptor Agonist and Antagonist". Involvement of clinician-investigators in clinical departments, such as Dr. Sumner in Project 1 or Dr. Kaufman in Project 5 are strengths of the program that will enhance its ability to identify human therapeutic interventions.

Progress in the first months of operation appears to be on target. Substantial synergy can be expected between the research programs specifically outlined in this collaborative agreement, and the larger intellectual framework formed the LSU Neuroscience Center of Excellence. Your role as director of both efforts is a vital feature that will ensure maximization of this synergy. In summary, I am most enthusiastic about this LSU-U.S. Army Cooperative Agreement, both for its specific merit and as a prototype mechanism for facilitating effective collaboration between academic and military institutions.

Best regards.

Sincerely,

Dennis Choi

Box 8111
660 South Euclid Avenue
St. Louis, Missouri 63110
(314) 362-7175 • FAX (314) 362-2826

525 EAST 68th STREET NEW YORK N Y 10021

THE NEW YORK HOSPITAL-CORNELL MEDICAL CENTER

FRED PLUM, M.D., CHAIRMAN
ANNE PARRISH TITZELL PROFESSOR OF NEUROLOGY
CORNELL UNIVERSITY MEDICAL COLLEGE
NEUROLOGIST-IN-CHIEF
THE NEW YORK HOSPITAL- CORNELL MEDICAL CENTER
(212) 746-6141
FAX (212) 746-8532

September 28, 1994

Nicholas G. Bazan, M.D., Ph.D.
LSU Neuroscience Center
2020 Gravier Street
Suite B
New Orleans, LA 70112-2234

Dear Dr. Bazan:

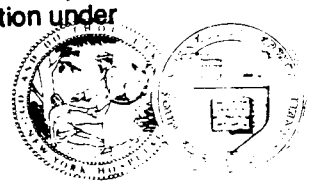
I am pleased to submit this reviewer's report of a Cooperative Agreement between the LSU Neuroscience Center and the US Department of the Army entitled, "Neural Response to Injury: Prevention, Protection and Repair" (henceforth designated as "Injury Study"). The agreement will span four years of effort by the LSU Center; this report describes progress obtained during its first year, extending from September 1, 1993 to August 31, 1994.

Nicholas G. Bazan, M.D., Ph.D. both directs the LSU Neuroscience Center of Excellence and serves as the Program Director of the Injury Study. In addition to Dr. Bazan's personal investigative efforts, seven additional study groups are engaged in research directly related to the Injury Study, as indicated in the administrative diagram attached to this report.

Dr. Bazan's outstanding personal and scientific qualities are the two most important factors in assuring the future success of the LSU-U.S. Army Cooperative Agreement. His leadership and intellectual "taste", as well as his joy in and dedication to brain science penetrate every aspect of the LSU Neuroscience Institute. His enthusiasm has spread to infect his colleagues and many other departments of the Medical School with his high scientific standards and integrity. His knowledge suffuses every dimension of basic neuroscience. His diplomacy and gentle handling of his staff creates their huge loyalty. His energy is contagious. Furthermore, he has the wonderful quality of scientific generosity: always ready to help and encourage others, he is entirely responsible for the continuously improving quality of young persons who are coming to LSU to learn and do important neuroscience.

In addition to the above, Dr. Bazan's specific research is internationally recognized as being of the highest caliber. His personal research contributions to the Injury Study during the past year reflects these high qualities in several ways. They have been published in the most competitively prestigious biomedical research journals. They also add new understandings to both the normal and potentially abnormal effects of the platelet-activating factor (PAF). PAF already is known to be a potent mediator of inflammatory and immune responses. What Bazan and his team now have found is that in low concentrations, PAF transmission may enhance memory and repair mechanisms in brain. Alternately, if released in excessively large concentrations or in combination with certain other molecules, PAF appears capable of causing immune-related tissue damage such as occurs with intense inflammation and/or the induction of genetic prostaglandin synthesis, a step that also may injure brain tissue. This fundamental research emphasizes the complexity and often bidirectional responses that may occur when injury strikes the brain. The results are important and illustrate the difficulties which must be overcome in establishing prevention, protection and repair of brain injuries.

Drs. Bazan and Prescott Deininger have succeeded in developing a series of transgenic mice expressing a dominant mutant of platelet derived growth factor (PDGF). Remarkably enough, the animals thus far have shown no major behavioral alteration under



normal developmental conditions. Their reaction to ischemia, seizures and other circumstances has not yet been tested.

Let me turn now to some of the other, supporting projects: **Drs. R. Benerman, D. Kline and A. Sumner** have made good progress in their studies of neurotrophic factors and other mechanisms in human and experimental neuromas resulting from blunt and crush nerve injuries. Basic fibroblast growth factor (bFGF) was the most prominent factor found in human post-nerve injury neuromas with other specific factors either absent or reaching only very low levels of concentration. More precisely analytic experiments await the analyses of fresh neuronal material from the experimental preparations.

Drs. Herbert Kaufman and Roger Benerman have made brilliant advances using confocal microscopy to examine the cellular details of the human retina. To a degree never before possible they have safely demonstrated in awake human subjects the acute pathophysiology of laser injuries to cornea and their early transformation into fibroblasts. Detailed identification of anterior chamber cells has been possible and current efforts are underway to examine at great magnification the optic disc itself. Ocular fungus and herpes infections can be identified immediately and without introducing foreign substances against the cornea or into the eye. Application of the tool should have an important place in clinically applied military medicine.

During the past year, the investigators also have pursued their earlier discovery that ambient chilling of monkeys latently infected with H. Simplex induces an acute recurrence of cutaneous herpes. Furthermore, chronic ingestion of the beta blocker, propanelol, has been found to ameliorate or prevent the active recurrence. Clinical trials of this important discovery must be pursued as it has important practical aspects.

During the year, the necessary work to establish and equip the glaucoma research laboratory was undertaken. Next year's report can be expected to provide research results from that laboratory.

Dr. Joseph Moerschbaecher and his colleagues in pharmacology have initiated preliminary studies on the influence of delta opoid agonists-antagonists on learning and antinociception. Somewhat surprisingly, the agent damps the CO_2 response of breathing but has no antinociceptive effect. The same investigator is analyzing how anxiogenic drugs affect dopamine neurons in the ventral tegmental area of the rodent brain.

In another preliminary approach, **Drs. H.W. Thompson et al** have initiated experiments passing retroviral gene carriers into the eye with externally applied eye drops, thereby developing a new approach to deliver protection against certain ophthalmologic infections or enhancing the potential success of corneal transplant.

Drs. Richard Bobbin and Charles Berlin, thanks to the DOD grant, have added an excellent postdoctoral student as well as important new equipment to their laboratory. The laboratory's principal subject of interest is to find mechanisms for preventing the audiologic damage produced by intense sound. In guinea pigs, this has been achieved by stimulating calcium-dependent mechanisms in cochlear neurons. In another study, the laboratory has found in human studies that during the delivery of loud, binaural sounds, men and women suppress the noise in opposite sided ears from each other.

The above individual achievements provide only a part of the considerable effort, enthusiasm and success that the U.S. Army grant has brought to the LSU Neuroscience Center of Excellence (NCE). The following steps forward can also be emphasized:

1) Morale in the LSU-NCE rides at high pitch, encouraging scientific collaboration and the generation of new ideas.

2) Funds have been granted to subsidize the necessary equipment and technical personnel to establish a brain bank. Presently, approximately 50 specimens are available in storage with the Center holding good clinical records of the preterminal illness.

3) A program of "starter" grants designed to assist young investigators in conducting merit-deserving, self designed research projects has been initiated.

4) A highly popular state-wide Graduate School outreach summer program has been successfully concluded, attracting a strong interest in neuroscience among gifted college students.

5) An interdisciplinary graduate program in neuroscience was initiated and strongly encouraged by the faculty during 1993-94. As a result, nearly all of the graduate students (including the new entering class) are of very good quality. Indeed, other participating departments say that the Neuroscience graduate students are the best among the LSU biological sciences programs.

Summary. Under the generous auspices of a U.S. Army Cooperative Agreement, the LSU Neuroscience Center of Excellence is not only thriving but headed for far greater future productivity than at any time in the past. The admirable success of the program depends heavily on the foresight, intelligence, creativity and energy of two outstanding scientists, Herbert Kaufman and, especially, Nicholas G. Bazan. Their achievements and those of their colleagues totally warrant continuation of support. Indeed, every indication is that their extramural, non-Army support will continue to grow, making the program stronger and stronger as the years elapse.

One serious problem remains - that of sufficient space in which to do the studies that Dr. Bazan and his colleagues already have conceived so well. Prompt attention to and effective application of must be given to the DOD funds already awarded to construct new research space which will greatly increase the LSU Neuroscience team's opportunities for creative discovery.

I and my colleagues on the External Advisory Board of the LSU Neuroscience Center of Excellence strongly endorse the quality and number of achievements that have come from the U.S. Army-LSU-NCE collaboration. Thanks to strong leadership for the Center and a high degree of internally high morale and interdependence within the Center, it can be anticipated that the Cooperative Agreement will have a major impact on national neuroscience research as well as the specific medical needs of the U.S. Army.

Sincerely,

Fred Plum

Fred Plum, M.D.

FP/moc

REPAIR AND REGENERATION OF PERIPHERAL NERVE DAMAGE

Project Directors:

Roger Beuerman, PhD
David Kline, MD
Austin Sumner, MD

Participating Scientists:

John England, MD
Leo Happel PhD
Daniel Kim, MD
Cheryl Weill, PhD

FOREWARD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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( ) In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources Commission on Life Science, National Research Council (NIH Publication no. 86-23, Revised 1985).

( ) For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45 CFR 46.

( ) In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

Investigator Signature

ANIMAL USE
20 SEPTEMBER, 1993, THROUGH JULY, 1994

DAMD17-93-V-3013

The experimental animals used during this period for the project, Neural Responses to Injury: Prevention, Protection, and Repair, **Subproject: Repair and Regeneration of Peripheral Nerve Damage**, are as follows:

Species	Number Allowed	Number Used	LSU IACUC #
Monkey	15	12	1059
Rabbit	50	40	1060

K. L. Sherman
Investigator Signature

INTRODUCTION

Functional regeneration of peripheral nerves following injury is a major military and civilian medical concern. However, some injuries do not heal without difficulty, and perhaps 50% of these result in a common complication. This impediment, a neuroma, developes at the site of injury. It is a disorganized mass of connective tissue that is not easily penetrated by regrowing axons. Surgical repair of severed nerves suffers from this complication as well. This grant project is designed to uncover the cell-signalling mechanisms underlying the formation of the neuroma, and to develop biological and surgical maneuvers to prevent neuroma formation.

The presence of growth factors and growth factor receptors was studied in neuroma tissue removed from 13 patients. The use of Western blots allowed the assay of several growth factors and growth factor receptors in one sample. Basic fibroblast growth factor was present in all tested samples. Platelet-derived growth factor ßß and transforming growth factor were found in small amounts. Epidermal growth factor and its receptor were only present in cells cultured from the neuroma. The polymerase chain reaction was used to determine that the message for transforming growth factor and its receptor was present in some neuroma tissue and cultured cells.

A series of primates with experimental bilateral nerve injury, crush and blunt transection is underway. Twelve animals have undergone surgery with one resected at 6 months. As predicted, the crush revealed moderate regrowth, while the nerve after blunt section had a clear neuroma and no functional recovery.

In other studies, development of the rabbit avulsion model and brachial plexus

stretch injury is in progress. Patch-clamp recording of neurite endings from within human neuroma specimens has focused on sodium channel activity. These have been labeled immunohistochemically, and physiological evidence for their function has shown similarities to channel properties in lower mammals.

Experiment 1: Development of primate model of neuroma.

Neuroma formation is a proliferative disease primarily involving fibroblastic cells. The precise role of these fibroblasts has not been identified; however, one of the technical objectives of this study has been to identify the fibroblast and the growth factor environment in which they are surviving. The development of this environment and the development of the neuroma has been at the heart of this proposal. To do this, it has been necessary to establish a primate model with experimental neuromas. Rodent models, although usable, regrow more aggressively than human peripheral nerves. The potential for regeneration of rodent peripheral nerves is much greater than that for primates, and it is not feasible to carry out the same type of manipulation in a rodent nerve with the expectation of consistent neuroma formation.

Fundamentally, the model that has been used throughout the study has been part of this laboratory for a number of years. However, this has been the first time that a developmental approach has been taken to study the neuroma combined with the molecular cell biology analysis of the cells and cellular environment.

The methodology for this study has been standardized in this laboratory with the outcome of been able to reliably establish neuromas from two types of surgical procedures: one is a blunt transection, and the other is a severe crush without

disturbing the continuity of the peripheral nerve. The Rhesus monkey has been used throughout for these experiments, and we have continued to use the adult animal.

All animal procedures are carried out in the operating suite of the Department of Animal Medicine here at the LSU Medical Center. Under anesthesia and using sterile techniques, sciatic nerve complexes of both legs are surgically exposed. Baseline-evoked nerve action potentials (NAP) and muscle action potential (MAP) are recorded for both tibial divisions using differential amplification and an oscilloscope. The animal then has each leg in turn fixated in a strain-gage measuring apparatus so that muscle contraction power of a single twitch and of a sustained titanic contraction of gastrocnemius-soleus muscles is recorded.

Following completion of these baseline studies, a severe lesion in continuity is created to one tibial nerve in one limb, and a blunt transecting lesion to the other tibial nerve in the other limb. Surgical soft tissue wounds are then closed, and the animals observed carefully until awake and able to regain an upright posture. In addition, the animals are observed on an every-other-day basis for problems in locomotion and related to the nerve injuries. To date, there have been no complications, and all the animals have been able to care for themselves, and there have been no infections associated with the wounds.

The attempt has been to create two different neuromas of variable severity. The real nerve function is to be reassessed, and the neuromas are to be removed for histological, physiological, and cell biological types of studies of the endoneurial fibroblasts at an interval of 12, 9, 6, and 3 months, as well as 1, 2, and 4 weeks.

To date, neuroma lesions have been created in 12 animals since this phase of

the work began in mid September of 1993. Each one of these animals has been tended by two neurosurgeons, and each one is essentially a day's procedure to carry out the electrophysiological mapping of the nerves and their muscle innervation. Two animals have succumbed to illness, most likely pneumonia, at 4 and 5 months postoperatively. Neuromas have formed in the nerves of each of their limbs, and in early histological view, the experimental design has been gained. Three other animals will be available for a 12-month study later this fall, three for a 6-month study early next year, and two for a 3-month study also this fall. It is planned to do one more 3-month animal at the end of the summer, and in September and October to do the 1-, 2-, and 4-week animals, two to three at each interval, which of course, will require more acute re-operation and reevaluation. The injury site from one 6-month animal has been reinvestigated. As predicted, the functional studies for the crush and blunt section models are considerably different. The nerve action potential (NAP) and muscle activation (MAP) were greatly recovered after the crush, and absent in the blunt section. The tissues, injury site, and proximal and distal segments will be analyzed separately using Western blots and PCR. The results from this experiment will form the fundamental core of understanding of the molecular events that are developed which will encourage the exaggerated growth of the neuroma, and then the part of the mechanism that sustains this growth.

The neuroma is a vascularized, encapsulated structure, which has a large extracellular matrix component. In the human neuroma studies, it has been found that fibroblast growth factor (bFGF) is the most prominent among the growth factors tested. However, it is felt that the early stage of the neuroma formation may be in fact

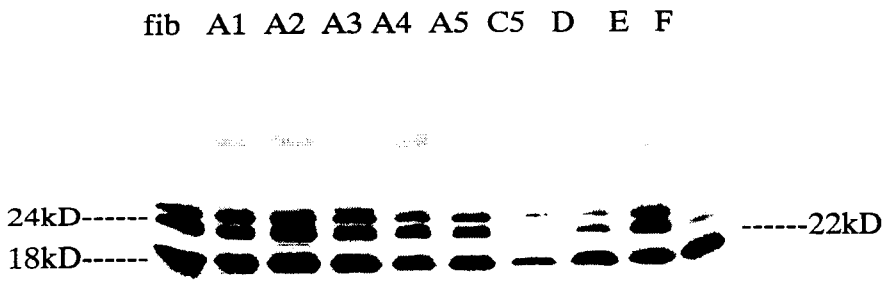
the most critical, and provide the insight into the future development. In these animals, particularly those at the early stages 1, 2, and 4 weeks, in addition to studies on growth factors, the role of cytokines, tumor necrosis factor, IL-1, and IL-6 will be examined. Certainly, the peripheral nerve injury will also involve inflammatory reaction occurring for some variable period of time. Macrophages, the principal producer of tumor necrosis factor, are likely to be prominent cell components of the early stage injury. Inflammatory cells and those of vascular origin would also be expected to be in the environment as new vessel formation begins.

Experiment 2: Western blot detection of growth factors in neuroma tissue.

The purpose of this study was to determine, in the fresh neuroma tissue, the presence of growth factors that may have a potential role in cell signalling in the development and maintenance of this tissue mass. Certainly it is clear that growth factors such as fibroblast growth factor and platelet-derived growth factor may be particularly important in the early stages of the wounding response and may help to direct part of the tissue reorganization in response to injury. On the other hand, transforming growth factor (TGF) has less of an affect on the mitotic potential of cells and, in fact, may even be somewhat antimitotic. However, TGF was originally considered to play a large role in the development of the matrix in that it can lead to increased production of both collagen and extracellular matrix proteoglycans.

In these studies, tissue was obtained from the operating room on an almost weekly basis and the tissue was used either for the development of cell cultures or for Western blots, and some specimens were used for PCR studies (Experiment 3).

Table I summarizes the results of the study. As indicated under tissue identification, more than one piece of neuroma tissue was usually obtained from each patient. The blots were run on mini-gel preparation with standards, either foreskin fibroblasts, A431, or the growth factor itself. As seen in Fig. 1, the results for basic FGF revealed one to three bands. Five isoforms of FGF are known, ranging in size from 18-29 kD; however, only the 18-24 kD bands were seen in the cells and tissues. Work in progress indicates that the amount of bFGF in some specimens may be as much as 1-5 μg. Epidermal growth factor does not appear to exert a role in the tissue *in vivo*. However, confluent, non-starved cultures of neuroma cells revealed a strong signal for the cytoplasmic domain of the EGF receptor. This was also confirmed by a quantitative flow cytometry method developed in this laboratory (1). Tumor necrosis factor (TNF-alpha) was not found by Western blots. However, in a study using ligated sciatic nerve in rat, TNF-alpha was positive. It will remain to be seen if a complex temporal expression of growth factor and cytokines interacts to govern the early development of the neuroma.



Western blot of bFGF of human neuroma tissues using 1ug/ml of monoclonal mouse anti human bFGF antibody (Transduction Laboratories). From left to right, fib is human foreskin fibroblast used as positive control (Transduction Laboratories); A2 is normal nerve; A3 is sural normal nerve; A1 and A4 to F are different samples of neuroma tissue. While the 18kD component of bFGF are present in all samples , there is the quantitative difference inother two components of 24kD and 22kD.

TABLE I

Tissue	Sex	Race	Age	Specimen	Specimen Date	EGFR	FGFRII	bFGF	PDGFBB	TGFBR2
1A	F		57	p.sc.neu	02-16-94		+		+	
1B	F		57	d.pe.neu	02-16-94		+		+	
2A	M	W	29	scar RN	07-28-93	-	-	++,1		
2B	M	W	29	lat. cord	07-28-93	-	-	+,1		
3A	M	W	24	scar RN	07-28-93	-	-	+,3		
4A	M			per neur	02-23-94		+		+	
4B	M			sci neur	02-23-94		+		+	
4C	M			sci scar	02-23-94		+		+	
5A	M	W	29	surN ner	01-19-94	-	+	++,4		
5B	M	W	29	tib. neur	01-19-94	-	-	++,4		
5C	M	W	29	A4-cell				++,3	++	+
5D	M	W	29	sci.neur	01-19-94	-	-	++,4	pro+	
6A	M		38	N. ner	01-12-94	+	+	+++,4		
6B	M		38	A2-cell					+	+
6C	M		38	neur	01-12-94	-	+	++,4		
6D	M		38	A-1 Cell				++,3	++	+
7A	F	W	34	neur	07-07-93			++,3		+
7B	F	W	34	neur	07-07-93			++,3		+
8A				neur			+		+	
9A	M	W	21	neur	03-11-93	-	+	++,3		
10A	M		24	C5	12-12-93	-	+	+,4		
10B	M		24	C6	12-12-93	+	+	++,4		
10C	M		24	C7	12-12-93	+	+	++,3		
11A	M		26	up trunk	12-08-93				+	
11B	M		26	mi trunk	12-08-93				+	
11C	M		26	C5	12-08-93				+	
11D	M		26	cell	12-08-93		+			
12A	M	W	38	up trunk	09-01-93	+/-	+	+,3		
13A	M	W	39	C6 C8	07-21-93					

p = proximal
d = distal
sc, sci = sciatic
pe, per = peroneal
sur = sural
up = upper

neur, neu = neuroma
lat = lateral
RN = radial nerve
N = normal
ner = nerve

+ = detected
- = not detected at the time
blank = data not available

Experiment 3: Growth factor message (mRNA) in human neuroma tissue and cells.

Neuroma specimens obtained directly from the operating room were either frozen in liquid nitrogen or minced and cultured in high glucose DMEM supplemented with 20% fetal bovine serum (FBS) and 1% antibiotic-antimycotic. The outer connective tissue covering was removed and the inner core of the neuroma cut into thin slabs to initiate cultures. Cultures have been passaged and stocks frozen as representative examples. Cultures were subsequently maintained in DMEM plus 10% FBS and 1% antibiotic-antimycotic. In these experiments there was no attempt to isolate Schwann cells from fibroblasts in these cultures since it was important to determine the contribution of these broadly to the growth factor environment of the human neuroma. Cell cultures were tested with the antibody to S 100 protein, which has specificity for Schwann cells. The results showed that the cultures were mixed cell types, fibroblasts, and Schwann cells. Oligonucleotide synthesis primers for the PCR were either taken from published sequences or chosen from published cDNA sequences by Oligoanalysis Software. The primers were synthesized by the LSU CORE Laboratory here at the LSU Medical Center. The total RNA from passage 2 neuroma cell cultures was extracted using Trizol (Gibco/BRL) reagent where the frozen neuroma specimens were ground up in liquid nitrogen and extracted by the guanidium/CsCl ultracentrifugation method. The integrity of the RNA was analyzed by agarose gel electrophoresis and quantitated spectrophotometrically. To prepare the cDNAs, 1 μg of total RNA was reverse transcribed in a final volume of 20 μl containing 2 μl of 10x PCR buffer, 1.2 μl of 25 mMol $MgCl_2$, 8 μl of 10 mMol dNTPs, 1.5 μl random hexamer, 20 units RNA Guard (Pharmacia), and 200 units of MoMuLV reverse

transcriptase. The mixture was incubated at 25°C for 10 min., 37°C 30 min., and heated to 95°C for 5 min., then ice chilled. For PCR, the cDNA mixture was incubated in a mixture containing 8 μl of 10x PCR buffer, 4.5 μl of 25 mMol $MgCl_2$, 2 μl each of 10 mMol specific primers and brought to 99 μl with sterile distilled water and overlayed with 100 μl of mineral oil. The mixture was heated to 94°C for 5 min. and 1 μl of Tag polymerase (5 units/μl) was added. Amplification was performed in an Omnigene thermal cycler at 94°C for 1.5 min., 50 to 70°C for 2 min., and 72°C for 2 min. for 4 cycles. Amplification was continued for 30 more cycles at 94°C for 1 min., 50 to 70°C for 1 min., and 72°C for 1 min. with the final extension at 72°C for 5 min. The PCR product was visualized on a 1% agarose gel. The PCR products were verified in the following fashion. The amplified fragments were purified with Centricon 100 μ-concentrators (Amicon) and redissolved in sterile water. Restriction endonuclease digestion with the appropriate enzyme 5 units/μg (New England Biolab) was performed at 37°C for 6 hours and fractionated on a 2% agarose gel. In addition, the "Hot Blot" was also used for confirmation of the products. Briefly, an internal 38 Oligomer was end labeled with gamma ^{32}P (3,000 Ci/mMol, NEN) by T4 kinase and purified by gel chromatography. From this mixture, 20 μl containing 1 x 10^{cpm} was incubated with 30 μl aliquots of PCR mixture and incubated at 94°C for 1 min., 43°C for 2 min., and 78°C for 2 min. then ice chilled. An aliquot of 30 μl was removed from the samples and was run on a 1 to 2% agarose gel, dried and exposed to Kodak X-OMAT film for 2 hours or overnight at room temperature. The results for tissue from two human neuromas and cells are seen in the accompanying table (Table II).

TABLE II

	Neuroma AL Tissue	Neuroma BT	Neuroma Cell Culture
TGF-b1	+	+	+
TGF-b2	-	-	+
TGF-b3	+	+	+
TGF-b3 receptor	+	+	+
FGF	+	+	+
FGF receptor	+	+	+
NGF	+	+	-
NGF receptor	+	+	-
GCSF			-
PDGF-A			+
PDGF-B			

TGF-b1: Transforming growth factor - beta 1
TGF-b2: Transforming growth factor - beta 2
TGF-b3: Transforming growth factor - beta 3
TGF-b3 receptor: Transforming growth factor - beta 3 receptor
FGF: Basic fibroblast growth factor
FGF receptor: Basic fibroblast growth factor receptor
NGF: Nerve growth factor
NGF receptor: Nerve growth factor receptor
GCSF: Granulocyte colony stimulating factor
PDGF-A: Platelet-derived growth factor - alpha
PDGF-B: Platelet-derived growth factor - beta

Although the transforming growth factors (TGF) were not readily detected by Western blots, it is clear that the mRNA was present not only in the cell cultures, but also in the beta-1, beta-2, beta-3 forms in the neuroma tissue taken from the operating room. As well, the receptor which could not be readily determined by Western blot was found by RTPCR. The presence of fibroblast growth factor (FGF) and its receptor were verified by this procedure. In addition, both nerve growth factor and its receptor were

found in the tissue from the neuroma but were not found in the neuroma cultures. At the present time, PDF-alpha has been tested in the neuroma cell culture and GCSF has not yet been tested. In addition, PDGFßß has yet to be tested by RTPCR but is expected to be present in at least some of the cell cultures some tissues as it was seen occasionally by the Western blot procedure.

Abstracts from these studies are appended. In addition, two papers are in progress, one focusing more on the PCR results, and another on the Western blot analysis of the human tissue series.

Experiment 4. Electrophysiological studies of membrane in neuromas.

Over the course of the past year methods have been developed to enzymatically digest and dissociate fibers in a human peripheral nerve neuroma. These methods represent modifications of those used by other investigators studying the membrane of axons in various mammalian species and have permitted access to the growing tips of neurites contained within the neuroma. Of 22 specimens obtained from nerve surgery 14 have been successfully treated with enzyme and triturated to expose the neurites and allow for patch-clamp recording. One problem encountered in this area has been the vast amount of connective tissue and scar present in the human neuroma which is not found in lower mammalian species. In 8 of the 22 specimens the scar tissue was too abundant to allow for sufficient fiber dissociation.

Of these 14 neuromas only 3 have thus far yielded successful patch-clamp recordings. This low yield was anticipated and is consistent with other laboratories. These conditions are acceptable since the information gleaned is so meaningful. The

recordings have been directed initially at studying sodium channels in an effort to relate information to other areas of this multidisciplinary investigation. The results, thus far, have shown that the sodium channels identified by immunocytochemistry and quantified by radioimmunoassay are indeed functional channels. They can be seen to be present in extraordinary concentrations as also indicated by immunocytochemistry and radioimmunoassay. In addition, these sodium channels show zero-voltage slope conductances and inactivation kinetics which are very similar to those of lower mammals. The channels are tetrodotoxin-sensitive like those of lower mammals.

Several problems have arisen and are presently being approached. First, the vast amount of connective tissue found in some of the neuromas has proven resistant to sufficient mechanical dissection to allow adequate enzyme digestion. Second, once enzymatic digestion has been completed an unidentified lipid appears in the bath solution. This lipid, which appears as minute droplets, has a tendency to plug micropipette tips and so preclude recording. Efforts are being directed to identifying this lipid in order to eliminate it and enhance the yield of successful recordings.

Thus far, an insufficient number of specimens has been studied to gain statistical significance. For this reason, codes which identify whether the neuroma was painful or not have not been broken and the study remains blinded. Thus, it is impossible to report the association of these findings with pain at this time.

Future studies are now being planned which would investigate the presence and characteristics of potassium channels found in neuromas. Once all of the channel populations have been identified and their distributions learned, it should be possible to describe the mechanism of altered membrane excitability in the growing neurites of

peripheral nerve neuromas.

CONCLUSION

The studies of this grant project are unique in their emphasis on the cellular mechanism for the development growth of peripheral nerve neuromas. Despite a large body of knowledge on peripheral nerve regeneration, there is little information on this debilitating consequence of peripheral nerve injury. Neuromas are the primary impediment to functional recovery of these injuries. The rigors of the military environment provide numerous opportunities of these injuries, which often leave the individual permanently debilitated.

The series of monkey studies will provide the basic knowledge of the cell-signalling factor controlling the development and growth of this tissue mass. The human studies are concerned with pain as a consequence of the neuroma, and the special feature of the nerve membrane of the growing neurites caught up in the neuroma. Information about growth factor and growth factor receptors of the human neuroma has been valuable. The disadvantage of these tissues is that they are mature neuromas; however, a number of them were painful. A preliminary study of an early rat neuroma showed the presence of TNF-alpha, a largely macrophage product, and this has not been seen in any of the human neuroma tissue.

Basic FGF was clearly the most widely seen and most abundant of all the growth factors in the human neuroma. Studies underway indicate the amount may be as high as 1 ng in the tissue. FGF may not actually be released within the tissue and probably serves as an autocrine or paracrine role. Immunohistochemical studies of

the cultured cells and neuroma tissue will show more clearly the cells involved. FGF has at least five isoforms and three were present in several tissue samples. However, the lower molecular weight components, 18 kD, was seen most frequently. Other investigators have shown that tissue cultured cells from many types of tumors are sensitive to FGF and produce FGF as well. Moreover, all FGF's have the potential to transform cells. Tissue culture studies of FGF and the FGF receptor will examine the expression of the protein and the receptor using a standard wound model. These results will be compared with Schwann cells and fibroblasts from normal nerve. Separation of the slower growing Schwann cells in culture can be carried out by inhibition of the fibroblasts. It was somewhat surprising that at least half of the population of cultured cells stained positively for S-100 protein. The tissue cultures were prepared from the inner collagenous core of the neuroma, and it was originally thought that most of these cells would be fibroblasts.

The origin of the cells in a neuroma is being explored using rodent models. These experiments will trace cell movements from the proximal and distal ends of an injury site. Cells will be tagged by microliter injection of a dye that binds to nucleic acids. These are nontoxic, and cells can often be followed after one or two divisions. Various procedures will test the potential for cells to move into a developing neuroma. Additionally, immunohistochemical verification of Schwann cells will be performed at the time of analysis. It should be feasible to combine these studies with small injection of particular cytokines and growth factors to determine their role in controlling cell fate in injuries.

Blocked regrowth of axons, as occurs with a neuroma, interferes with the

relationship of the neuron cell body and its peripheral target time. Neuron survival after long-term neuroma may be compromised, and the potential for regrowth after surgical intervention may be limited for these reasons. It is anticipated that the viability of motorneuron will be examined in rodents after experimental neuromas by in situ hybridization of trk receptor. This should lead to an understanding of the temporal course of effects on the motorneuron, and if there is a specific time period for normal axon and growth, and the injury-like effects on the spinal motorneuron.

The neurophysiological studies of the entrapped neurites will make use of the series of monkey neuromas to examine the time course of the development of the abnormal ion channels. In addition, the immunohistochemical studies of sodium channels will provide structural evidence for the changes.

SECTION II
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TITLE: GROWTH FACTORS AND RECEPTORS IN CELLS FROM HUMAN NEUROMAS

ABSTRACT: (single space):

A neuroma is a disorganized mass of connective tissue and axons that causes unsatisfactory peripheral nerve regeneration and often pain. The cellular mechanisms leading to the exaggerated and haphazard fibroblastic proliferation and extracellular matrix production characteristic of neuromas are unknown. Growth factors could be important in the cellular stimulation that leads to neuroma formation and growth. We removed 23 neuroma samples from 12 patients (ages 21-57 years); specimens were either assayed for the presence of growth factors and their receptors or prepared for tissue culture. Methods included Western blots (foreskin fibroblasts or the specific growth factor were used as controls) and the polymerase chain reaction (PCR). Basic fibroblast growth factor (bFGF) was present in the largest amount ($62 \pm 0.11\%$ of control, mean$\pm$SE) and was found in samples from all 12 patients, as well as in neuroma cell cultures. The bFGF receptor was found in small amounts in 7/12 patients. Platelet-derived growth factor-BB (PDGF-BB) was present in small amounts in samples from 5/12 patients. Epidermal growth factor and its receptor were present only in cultured neuroma cells. PCR demonstrated messages (mRNA) for transforming growth factor and its receptor in tissue-cultured cells and in 4/12 patients. The results suggest an important role for fibroblast growth factor in cell signaling within the neuroma. These signals appear to be essential for the proliferation of connective tissue seen in most neuromas. Future studies will seek to determine how fibroblast growth factor acts to stimulate growth of a neuroma and possible approaches to modulation of these mechanisms.

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EPIDERMAL GROWTH FACTOR AND FIBROBLAST GROWTH FACTOR IN HUMAN NEUROMA TISSUE. R. Beuerman*, S. Zhao, H. Tran, S. Ma, D. Kim, D. Kline, LSU Eye Center and Dept. of Neurosurgery, LSU Neuroscience Center, LSU Medical School, New Orleans, LA 70112

The formation of neuromas after injury or surgical repair of peripheral nerves interferes with the establishment of functional connections by the regenerating axons. The purpose of this study was to determine the types of growth factors and growth factor receptors in human neuroma tissue and in cultured cells from these tissues. Tissues obtained from five patients at the time of surgery performed 8-10 months after trauma to the brachial plexus was frozen in liquid nitrogen and small pieces were used to establish primary cultures. Western blots for EGFR, FGF, and bFGFR were carried out on cells and fresh tissue using antibodies for EGFR (cytoplasmic), bFGF, and FGFR-1. The results showed that EGFR was present in confluent cultured cells but not in the tissue. The 18kD form of bFGF was present in cells and all tissues; the higher molecular weight forms were seen in three of the tissues. FGFR-1 was found in the cultured cells and, less abundantly, in the tissues. The cultured cells stained positively for S-100 protein, suggesting that they were Schwann cells. PDGF-bb was found in the cultured cells but not in any of the tissues. The results suggest that FGF could be acting in an autocrine fashion on cells in neuromas and that this growth factor may have a role in the development of this condition.

Supported in part by DAMD17-93-V-3013.

KEY WORDS: (see instructions p. 4)

1. Neuroma
2. Peripheral nerve regeneration
3. Growth factor
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